

ATTORNEY'S DOCKET NO.: 24795

U.S. DEPARTMENT OF COMMERCE, PATENT AND TRADEMARK OFFICE		DATE: <u>28</u> September 2001 (<u>28</u> 09 2001)
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. AGENT FOR PROSECUTION: <u>09/29/2001 848</u> Not Yet Assigned
INTERNATIONAL APPLICATION NO.: PCT/FR00/00818	INTERNATIONAL FILING DATE: 31 March 2000 (31.03.00)	PRIORITY DATE CLAIMED: 02 April 1999 (02.04.99)
TITLE OF INVENTION: COSMETIC COMPOSITION COMPRISING AT LEAST ONE SUBSTANCE PROMOTING THE FORMATION OF CONNEXIN, USE AND METHOD OF COSMETIC TREATMENT		
APPLICANT(S) FOR DO/EO/US: NIZARD, Carine; PROVOST, Nicolas; VIRON, Cécile; KRZYCH, Valérie; SAUNOIS, Alex		
Applicant hereby submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<p>1. <u>X</u> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <u> </u> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <u>X</u> This express request to begin national examination procedures (35 USC 371(f) The submission must include items(5), (6), (9) and (21) indicated below.</p> <p>4. <u>X</u> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</p> <p>5. <u>X</u> A copy of the International Application as filed (35 U.S.C. 371(c)(2)):</p> <p style="margin-left: 40px;">a. <u>X</u> is transmitted herewith (required only if not transmitted by the International Bureau).</p> <p style="margin-left: 40px;">b. <u> </u> has been communicated by the International Bureau.</p> <p style="margin-left: 40px;">c. <u> </u> is not required, as the application was filed in the United States Receiving Office (RO/US)</p> <p>6. <u>X</u> A English translation of the International Application as filed (35 U.S.C. 371(c)(2)).</p> <p>7. <u>X</u> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <p style="margin-left: 40px;">a. <u> </u> are attached hereto (required only if not transmitted by the International Bureau).</p> <p style="margin-left: 40px;">b. <u> </u> have been communicated by the International Bureau.</p> <p style="margin-left: 40px;">c. <u> </u> have not been made; however, the time limit for making such amendments has NOT expired.</p> <p style="margin-left: 40px;">d. <u>X</u> have not been made and will not be made.</p> <p>8. <u> </u> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <u> </u> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</p> <p>10. <u> </u> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p>		
ITEMS 11 to 20 BELOW CONCERN OTHER DOCUMENT(S) OR INFORMATION INCLUDED:		
<p>11. <u>X</u> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <u> </u> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <u>X</u> A FIRST preliminary amendment.</p> <p>14. <u> </u> A SECOND or SUBSEQUENT preliminary amendment</p> <p>15. <u> </u> A substitute specification.</p> <p>16. <u> </u> A change of power of attorney and/or address letter.</p> <p>17. <u> </u> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter2 and 35 USC 1821 - 1825</p> <p>18. <u> </u> A second copy of the published international application under 35 USC 154(d)(4)</p> <p>19. <u> </u> A second copy of the English language translation of the international application under 35 USC 154(d)(4)</p> <p>20. <u>X</u> Other items or information:</p>		
<p>TRANSMITTAL FORM; FEE CALCULATION; INTERNATIONAL PUBLICATION WO 00/59466; INTERNATIONAL PUBLICATION DATE 12 OCTOBER 2000 WITH VERIFIED ENGLISH TRANSLATION CONSISTING OF 33 PAGES INCLUDING; 23 PAGES TEXTUAL SPECIFICATION, 4 PAGES OF 25 CLAIMS; 1 PAGE CONTAINING THE ABSTRACT; 5 SHEETS DRAWINGS; PRELIMINARY AMENDMENT; UNEXECUTED INVENTOR'S DECLARATION; PCT/PEA/416 AND PCT/PEA/409 INTERNATIONAL PRELIMINARY EXAMINATION REPORT; PCT/ISA/220 AND PCT/ISA/210 INTERNATIONAL SEARCH REPORT; INFORMATION DISCLOSURE STATEMENT TRANSMITTAL LETTER; INFORMATION DISCLOSURE STATEMENT; PTO FORM 1449 WITH 5 REFERENCES; PCT/RO/101 REQUEST; PCT/IB/301 NOTIFICATION OF RECEIPT OF RECORD COPY; PCT/IB/304 NOTIFICATION CONCERNING SUBMISSION OF PRIORITY DOCUMENT; PCT/IB/308 NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES; PCT/IB/332 INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION.</p>		

ATTORNEY'S DOCKET NO: 24795

U.S. APPLICATION NO. (if known) not yet assigned 09/937848	INTERNATIONAL APPLICATION NO. PCT/FR00/00818	DATE: 28 September 2001 (28 09 2001)
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17. <u>x</u> The following fees are submitted: Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO:\$860.00 International preliminary examination fee paid to USPTO (37 CFR 1.482).....\$690.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....\$710.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....\$1000.00 International preliminary examination fee (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....\$ 100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =	<u>CALCULATIONS</u> \$860.00 \$ 860.00	<u>PTO USE ONLY</u>
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Surcharge of \$130.00 for furnishing the oath or declaration later than <u> 20 </u> <u> 30 </u> months from the earliest claimed priority date (37 CFR 1.492(e)).	\$	
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CLAIMS	NO. FILED	NO. EXTRA	RATE		
TOTAL	33 -20=	13	X \$ 80.00	\$	234.00
INDEPENDENT	5 -3=	2	X \$ 80.00	\$	160.00
Multiple dependent claims(s) (if applicable)			+ \$260.00	\$	0.00
TOTAL OF ABOVE CALCULATIONS =				\$	1,254.00
Reduction by 1/2 for asserting small entity, if applicable. (Note 37 CFR 1.9, 1.27, 1.28).				\$	0.00
SUBTOTAL =				\$	1,254.00
Processing fee of \$130.00 for furnishing the English translation later than <u> 20 </u> <u> 30 </u> months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	0.00
TOTAL NATIONAL FEE =				\$	1,254.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	0.00
TOTAL FEES ENCLOSED =				\$	1,254.00
				Amount to be: refunded _____ charged _____	\$ _____ \$ _____

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09/937848	PCT/FR00/00818	

a. ☒ One check in the amount of \$1,254.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 14-0112 in the amount of \$_____ to cover the above fees. (A duplicate copy of this sheet is enclosed.)

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0112.

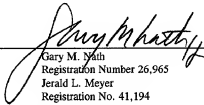
d. Fees are to be charged to a credit card ____ WARNING: Information on this form may become public ____ Credit Card Information should not be included on this form. ____ Provide credit card information and authorization on PTO-2038 ____

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed to request that the application be restored to pending status.

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Rev. 02/98

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

09/937848

In re Application of:

1) NIZARD, Carine; 2) PROVOST, Nicolas; 3) VIRON, Cécile;
4) KRZYCH, Valérie, 5) SAUNOIS, Alex

International Application No. PCT/FR00/00818

Serial No. NOT YET ASSIGNED

International Filing Date: 31 March 2000 (31.03.00)

Filed: September 28, 2001

COSMETIC COMPOSITION COMPRISING AT LEAST ONE SUBSTANCE
PROMOTING THE FORMATION OF CONNEXIN, USE AND METHOD OF COSMETIC
TREATMENTTRANSMITTAL LETTERCommissioner of Patents
Washington, D.C. 20231

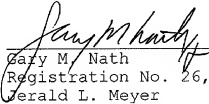
Sir:

Submitted herewith for filing in the U.S. Patent and Trademark
Office is the following:

- (1) Transmittal Letter
- (2) Transmittal Letter To U.S. Designated/Elected Office (DO/EO/US)
Concerning Filing under 35 U.S.C. 371
- (3) International Publication No: WO 00/59466
International Publication Date: 12 October 2000 (12.10.00)
with verified translation consisting of 33 pages including:
 - 23 pages textural specification,
 - 4 pages of 25 claims
 - 1 page containing the Abstract
 - 5 sheets of drawings
- (4) Preliminary Amendment
- (5) Unexecuted Inventor's Declaration
- (6) PCT/IPEA/416 and PCT/IPEA/409 International Preliminary
Examination Report
- (7) PCT/ISA/220 and PCT/ISA/210 International Search Report
- (8) Information Disclosure Statement Transmittal Letter
- (9) Information Disclosure Statement
- (10) PTO Form 1449 with 5 references
- (11) PCT/RO/101 Request
- (12) PCT/IB/301 Notification of Receipt of Record Copy
- (13) PCT/IB/304 Notification Concerning Submission of Priority Document
- (14) PCT/IB/308 Notice Informing the Applicant of the
Communication of the International Application to the
Designated Offices
- (15) PCT/IB/332 Information Concerning Elected Offices Notified
Of Their Election
- (16) Check No. 15574 \$1,254.00 for Government Filing Fee
- (17) Postcard for early notification of serial number.

Respectfully submitted,
NATH & ASSOCIATES PLLC

By:


Gary M. Nath
Registration No. 26,965
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Customer No. 20529

Date: September 28, 2001

BOX PCT

Attorney Docket No. 24795

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

1) NIZARD, Carine; 2) PROVOST, Nicolas; 3) VIRON, Cécile;
4) KRZYCH, Valérie, 5) SAUNOIS, Alex

International Application No. PCT/FR00/00818

Serial No. NOT YET ASSIGNED

International Filing Date: 31 March 2000 (31.03.00)

Filed: September 28, 2001For: **COSMETIC COMPOSITION COMPRISING AT LEAST ONE SUBSTANCE
PROMOTING THE FORMATION OF CONNEXIN, USE AND METHOD OF COSMETIC
TREATMENT****PRELIMINARY AMENDMENT**Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examining on the merits and calculating the filing fee for the national phase application filed herewith, please enter the following amendments:

IN THE CLAIMS:

Please cancel claims 1 through 25 and submit the following new set of claims 26 to 58, per Attachement A, without prejudice or disclaimer.

REMARKS

The above amendments have been made to remove multiple dependencies from the claims and to conform them to U.S. practice. No new matter has been added. Pursuant to the new rules implementing the AIPA, a clean copy of the new claims is attached.

Respectfully submitted,

NATH & ASSOCIATES PLLCBy: Gary M. Nath
Registration No. 26,965
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customer no. 20529Date: September 28, 2001
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GMN/dd:AMENDpreml.AIPA

ATTACHMENT A

20

IN THE CLAIMS

Please cancel all pending claims 1 through 25 and enter new claims 26 to 58, in lieu thereof without prejudice or disclaimer as follows :

25

CLAIMS

30

26. A cosmetic composition comprising, as an active ingredient, at least one substance which promotes the intercellular communication of skin cells, particularly keratinocytes, fibroblasts and skin preadipocytes, in a cosmetically acceptable excipient.

27. The composition of claim 26, wherein said substance promotes intercellular communication via the gap junctions of skin cells, particularly keratinocytes, fibroblasts and skin preadipocytes.

35

28. The composition of claim 26, wherein said substance which promotes intercellular communication promotes the formation of connexin.

29. The composition of claim 26, wherein said substance which promotes intercellular communication comprises at least one lipid extract of the alga *Skeletonema*.

30. The composition of claim 29, wherein said lipid extract of the alga *Skeletonema* is a lipid extract of the alga *Skeletonema costatum*.

31. The composition of claim 30, wherein said lipid extract is a total lipid extract.

32. A cosmetic composition comprising, as an active ingredient at least one lipid extract of the alga *Skeletonema*, obtained by extracting the alga *Skeletonema* with an alcoholic solvent selected from the group consisting of isopropanol, ethanol and methanol.

33. The composition of claim 32, wherein the lipid extract is obtained by extracting the alga with isopropanol.

34. The composition of claim 32, wherein the lipid extract is obtained by extracting the alga with ethanol.

35. The composition of claim 32, wherein the extraction is performed under reflux.

36. The composition of claim 32, wherein the alga is frozen before being extracted with the alcoholic solvent, the freezing being effected at a temperature of between about -40°C and -20°C and for a period of time ranging between about 1 and 7 days.

37. The composition of claim 36, wherein the frozen alga is immersed directly in the heated alcoholic solvent.

38. The composition of claim 32, wherein the lipid algal extract is obtained after the following series of steps:

- a) the alcoholic solvent is alkalized to a pH ranging between 10 and 14.
- b) the insoluble materials are removed from the aqueous-alcoholic phase,
- c) distilled water is added to the aqueous-alcoholic phase,
- d) the solution obtained is subjected to liquid-liquid extraction with an apolar solvent immiscible with the aqueous-alcoholic phase.
- e) the phase containing the apolar solvent is removed,
- f) the aqueous-alcoholic phase recovered after removal of the phase containing the apolar solvent is acidified to a pH ranging between 1 and 3.
- g) the solution obtained after acidification is subjected to liquid-liquid extraction with an apolar solvent immiscible with the aqueous-alcoholic phase.

- h) the aqueous-alcoholic phase is removed, and
- i) the phase containing the apolar solvent recovered after removal of the aqueous-alcoholic phase is evaporated to give an oil free of apolar solvent, this oil being the target lipid extract of said alga.

5 39. The composition of claim 32, wherein the extract is obtained by extraction with supercritical CO₂.

40. The composition of claim 32, wherein, before any extraction operation, the alga is macerated in the alcoholic solvent at room temperature, for a period of time ranging between about 5 minutes and 80 minutes.

10 41. The composition of claim 32, wherein the amount of alcoholic solvent used is between about 0.1 liter and 20 liters of solvent, per 100 g of alga, expressed by dry weight of alga.

42. The composition of claim 32, wherein the extraction is performed under an inert atmosphere.

15 43. The composition of claim 32, wherein the extraction is performed under an inert atmosphere comprising or consisting essentially of a nitrogen-saturated atmosphere.

44. The composition of claim 32, comprising from about 0.01% to 10% by weight of said lipid extract of the alga *Skeletonema*, based on the total weight of the composition.

20 45. The composition of claim 32, comprising from about 0.1% to 3% by weight of said lipid extract of the alga *Skeletonema*, based on the total weight of the composition.

25 46. The composition of claim 44, wherein the alga *Skeletonema* is the alga *Skeletonema costatum*.

47. The composition of claim 26, wherein said substance which promotes the intercellular communication of skin cells is boldine.

48. The composition of claim 47, comprising from about 0.001% to 10% by weight of boldine, based on the total weight of the composition.

30 49. A method of cosmetic skin care, comprising the application, to the skin areas of a person in need thereof, of an effective amount of at least one substance promoting intercellular communication for obtaining an anti-ageing effect on said skin areas, for improving the firmness and elasticity of the skin, for delaying the appearance of wrinkles or for reducing their depth.

50. The method of claim 49, wherein said substance promotes intercellular communication via the gap junctions of keratinocytes, fibroblasts and skin preadipocytes.

5 51. The method of claim 49, wherein the substance which promotes intercellular communication promotes the formation of connexin.

52. The method of claim 49, wherein the substance which promotes intercellular communication promotes the formation of connexin 43.

53. The method of claim 49, wherein said substance comprises at least one lipid extract of the alga *Skeletonema*, said lipid extract being obtained by liquid-
10 liquid extraction between an alkalized and then acidified alcohol and an apolar solvent immiscible with the aqueous-alcoholic phase.

54. The method of claim 49, wherein said substance is boldine.

55. The method of claim 49, wherein said substance is presenting cosmetic composition comprising from about 0.001% to 10% by weight of said substance,
15 based on the total weight of the cosmetic composition.

56. A method of promoting and/or increasing the activity of a cosmetic agent acting directly in the cell or via intracellular second messengers, comprising the application, simultaneously with or prior to that of said cosmetic agent, to the skin areas of a person in need thereof, of an effective amount of at least one substance
20 promoting intercellular communication.

57. A method of promoting and/or increasing the activity of a cosmetic agent acting directly in the cell or via intracellular second messengers, comprising the application, simultaneously with or prior to that of said cosmetic agent, to the skin areas of a person in need thereof, of an effective amount of boldine.

25 58. The method of claim 57, wherein said boldine is present in a cosmetic composition comprising from about 0.001% to 10% by weight of boldine, based on the total weight of the cosmetic composition, said cosmetic composition comprising a cosmetically acceptable excipient.

5/psls

Cosmetic composition comprising at least one substance promoting the formation of connexin, use and method of cosmetic treatment

5 The invention relates essentially to a cosmetic composition comprising at least one substance which promotes the intercellular communication of skin cells, particularly keratinocytes, fibroblasts and skin preadipocytes, and especially the formation of connexin, to its use and to a method of cosmetic treatment.

10 The search for means of combating human ageing currently receives the attention of a large number of researchers. This is particularly the case in the field of cosmetics, where attempts are made to combat, or at least slow down, the appearance of the esthetic and physiological effects of skin ageing, such as wrinkles, loss of elasticity of the skin, loss of color, etc.

15 Ageing of the human species is characterized by numerous disorders affecting the living tissues. The signs of this natural process are easily detectable on an organ such as the skin (Montagna, W. & Carlisle, K. (1990). Structural changes in ageing skin. British Journal of Dermatology, 122(35), 61-70). Skin ageing is a combination of two phenomena, namely an intrinsic (genetic) cellular process associated with so-called extrinsic ageing, which groups together environmental aggressions (Grove, G.L. (1989). Physiologic changes in older skin. Clinics in
20 geriatric medicine, 5(1), 115-125). The effects of senescence on skin tissue are visible mainly in the formation of wrinkles. These reflect the substantial changes which take place in the dermis and the epidermis. Tissue homeostasis, which is important for the skin, is modified in the course of ageing. The gap junctions participate in the regulation of cell homeostasis, so their role in maintaining the
25 physiological equilibrium of the skin is important.

The inventors have demonstrated an ageing effect on the functional capacity of cells to communicate with one another, especially via the gap junctions (GJ), and also an ageing effect on the amount of connexin 43 present in the cells. In fact, they have demonstrated that the functional capacity of cells to communicate with one
30 another, especially via the gap junctions (GJ), decreases with age. They have also demonstrated that the amount of connexin 43 decreases with age. The inventors have therefore shown the value of using substances which promote intercellular communication, especially via the GJ, for combating the signs of skin ageing and particularly for slowing down their appearance. This represents a new means of
35 combating skin ageing, particularly wrinkles and the loss of elasticity of the skin.

The gap junctions (GJ) are transmembrane protein structures which allow small molecules (<1000 Da) to pass between two cells (Watt, F.M. (1991). Intercellular communication via gap junctions. In L.A. Goldsmith (Eds.), Physiology, biochemistry, molecular biology of the skin (pp. 857-859). New York, Oxford: Oxford University Press). These hexameric structures are called connexons, which are themselves formed from connexins.

When an intercellular contact is established, the connexons of one cell align end-to-end with those of the adjacent cell to form a junctional channel (Goodenough, D.A., Golier, J.A. & Paul, D.L. (1996). Connexins, connexons and cellular communication. Annual Review of Biochemistry, 65, 475-502). These gap junctions (GJ) make it possible to maintain the cell and tissue homeostasis. Connexins form part of a family of proteins each having tissue specificities. Connexin 43 is the majority protein in keratinocytes (Salomon, D., Saurat, J.-H. & P.M. (1988). Cell-to-cell communication within intact human skin. Journal of Clinical Investigation, 82, 248-254). In skin the GJ are present in all the layers of the epidermis except the stratum corneum.

Furthermore, it is known that molecules which affect the way cells function can do this either by entering the cells or by not entering the cells but binding to receptors present on the plasma membrane, thus triggering a series of reactions which culminate in the release of small molecules, called "second messengers", in the cytoplasm. These small second messengers, for example cAMP (cyclic adenosine 3',5'-monophosphate), circulate from one cell to the next via the GJ, thereby transmitting the information from cell to cell. The same applies to small molecules which enter the cells and are capable of circulating from one cell to the next via the GJ. Thus, by increasing the intercellular communication, especially via the GJ, the inventors have developed a novel means of promoting and/or increasing the activity of a cosmetic agent.

The inventors have also shown that, surprisingly, the use of boldine or, independently, the use of lipid extracts of the alga *Skeletonema costatum* makes it possible to restore intercellular communication, especially via the GJ, and also to increase the level of connexin 43 present in skin cells, especially keratinocytes, fibroblasts and preadipocytes.

Thus the main object of the present invention is to solve the new technical problem which consists in the provision of a novel solution for improving the efficacy of cosmetic compositions and particularly for combating, or at least

slowing down, the appearance of the esthetic and physiological effects of skin ageing, such as wrinkles, loss of elasticity of the skin, loss of color, etc.

According to the present invention, this technical problem has been solved for the first time, in a surprising and non-obvious manner, by the discovery that a substance which promotes the intercellular communication of keratinocytes, fibroblasts and skin preadipocytes makes it possible to provide a cosmetic composition which has improved properties, particularly for combating, or at least slowing down, the appearance of the esthetic and physiological effects of skin ageing, such as wrinkles, loss of elasticity of the skin, loss of color, etc.

Furthermore, the present invention has afforded the discovery, again in an unexpected and non-obvious manner, that a substance which promotes the formation of connexin, particularly connexin 43, makes it possible to prepare a cosmetic composition of enhanced efficacy, particularly for combating, or at least slowing down, the above-mentioned appearance of the esthetic and physiological effects of skin ageing.

According to the present invention, it has also been discovered, in an unexpected and non-obvious manner, that on the one hand boldine and on the other hand, independently, a lipid extract of the alga *Skeletonema*, especially the alga *Skeletonema costatum*, and particularly a total lipid extract of said alga, promote intercellular communication via the gap junctions of skin cells, particularly keratinocytes, fibroblasts and skin preadipocytes, and thus makes it possible to prepare a cosmetic composition of enhanced efficacy, particularly for combating or slowing down the appearance of the esthetic and physiological effects of skin ageing or for combating hyperadiposis.

Thus, according to a first feature, the present invention relates to a cosmetic composition which is characterized in that it comprises, as an active ingredient, at least one substance which promotes the intercellular communication of skin cells, particularly keratinocytes, fibroblasts and skin preadipocytes.

In one particular embodiment of the invention, the composition is characterized in that said substance promotes intercellular communication via the gap junctions of skin cells, particularly keratinocytes, fibroblasts and skin preadipocytes.

In another advantageous embodiment of the invention, the composition is characterized in that said substance which promotes intercellular communication promotes the formation of connexin, particularly connexin 43.

In yet another advantageous embodiment of the invention, the above-mentioned composition is characterized in that said substance which promotes intercellular communication comprises at least one lipid extract of the alga *Skeletonema*, especially the alga *Skeletonema costatum*, and particularly a total lipid extract of said alga.

It is pointed out that the alga *Skeletonema*, particularly *Skeletonema costatum* (called SKC in the remainder of the document), is a well-known single-cell alga of the phylum Chlorophytes, the branch Chrysophycophytes, the class Diatomophyceae and the order Centrales. Diatomophyceae are very widespread in fresh, salt or brackish waters. The life of the species of this class can be planktonic or benthic. The protoplasm is enclosed in a siliceous frustule. *Skeletonema costatum* (SKC) is a cosmopolitan and usually marine species, which is frequently found to be associated with the phytoplanktonic efflorescences of coastal waters.

In one advantageous embodiment of the invention, this lipid extract is characterized in that it is obtained by extracting the alga *Skeletonema* with an alcoholic solvent selected from the group consisting of isopropanol, ethanol and methanol.

In another advantageous variant, the extraction is performed under reflux.

In another advantageous variant, the alga is frozen before being extracted with the alcoholic solvent, the freezing preferably being effected at a temperature of between about -40°C and -20°C and for a period preferably of between about 1 and 7 days.

In another advantageous variant, the frozen alga is immersed directly in the heated alcoholic solvent. The thermal shock in fact makes it possible to facilitate the decantation of the silica (originating from the skeleton of the algal cells).

In another advantageous variant, in the case of extraction with an alkalinized alcohol, the above-mentioned algal extract is obtained after the following series of steps:

- a) the alcoholic solvent is alkalinized to a pH of between 10 and 14, preferably to a pH of 13, for example with aqueous sodium hydroxide solution or aqueous potassium hydroxide solution,
- b) the insoluble materials are removed from the aqueous-alcoholic phase,
- c) distilled water is added to the aqueous-alcoholic phase,
- d) the solution obtained is subjected to liquid-liquid extraction with an apolar solvent immiscible with the aqueous-alcoholic phase, for example heptane,

hexane or cyclohexane,

- e) the phase containing the apolar solvent is removed,
- f) the aqueous-alcoholic phase recovered after removal of the phase containing the apolar solvent is acidified to a pH of between 1 and 3, preferably to a pH of 2, for example with aqueous sulfuric acid solution or aqueous hydrochloric acid solution,
- g) the solution obtained after acidification is subjected to liquid-liquid extraction with an apolar solvent immiscible with the aqueous-alcoholic phase, for example heptane, hexane or cyclohexane,
- h) the aqueous-alcoholic phase is removed, and
- i) the phase containing the apolar solvent recovered after removal of the aqueous-alcoholic phase is evaporated to give an oil free of apolar solvent, this oil being the target extract.

- The use of an alkalized and then acidified alcohol affords an extract with visual and olfactory characteristics that are acceptable in cosmetic compositions (yellow coloration and acceptable odor).

In another advantageous variant, the above-mentioned extract is obtained by extraction with supercritical CO₂.

- In yet another advantageous variant, before any other extraction operation, the alga is macerated in the alcoholic solvent at room temperature, preferably for a period of between about 5 minutes and 80 minutes and particularly preferably for a period of between about 20 minutes and 40 minutes.

- In yet another advantageous variant, the amount of alcoholic solvent used is between about 0.1 liter and 20 liters of solvent, preferably between about 2 liters and 10 liters of solvent, per 100 g of alga, expressed by dry weight of alga.

In yet another advantageous variant, the extraction can be performed under an inert atmosphere, preferably a nitrogen-saturated atmosphere. This makes it possible in particular to avoid pronounced oxidative degradation of the active molecules.

- This lipid extract is preferably packaged under an inert gas, such as nitrogen, in order to protect the active molecules.

- In yet another advantageous variant, the above-mentioned composition is characterized in that it comprises from about 0.01% to 10% and particularly from about 0.1% to 3% by weight of said lipid extract of the alga *Skeletonema*, especially the alga *Skeletonema costatum*, based on the total weight of the final

composition.

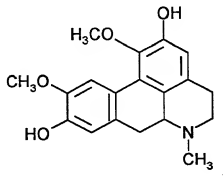
According to a second feature, the present invention further relates to the use of at least one substance which promotes the intercellular communication of keratinocytes, fibroblasts and skin preadipocytes as a cosmetic agent, optionally in the presence of a cosmetically acceptable vehicle.

In one particular embodiment of the use, the latter is characterized in that said substance promotes intercellular communication via the gap junctions of keratinocytes, fibroblasts and skin preadipocytes.

In another particular embodiment of the invention, the substance which promotes intercellular communication promotes the formation of connexin, particularly connexin 43.

In another embodiment of the invention, the substance comprises at least one lipid extract of the alga *Skeletonema*, especially the alga *Skeletonema costatum*, and particularly a total lipid extract of said alga, especially as defined above. Advantageously, this above-mentioned extract is obtained by liquid-liquid extraction between an alkalinized and then acidified alcohol and an apolar solvent immiscible with the aqueous-alcoholic phase, for example heptane, hexane or cyclohexane.

In another embodiment of the invention, the substance which promotes the intercellular communication of skin cells is boldine, a product of the following formula:



the compositions of the invention which contain said boldine advantageously containing it in an amount of about 0.001 to 10% and advantageously of 0.01 to 1% by weight.

According to a third feature, the present invention also covers a method of promoting and/or increasing the activity of a cosmetic agent acting directly in the cell or via intracellular second messengers, characterized in that it comprises the application, simultaneously with or prior to that of said cosmetic agent, to the

appropriate skin areas of a person in need thereof, of an effective amount of at least one substance promoting intercellular communication, particularly a substance promoting intercellular communication as defined above.

- According to a fourth feature, the present invention also covers a method of
- 5 cosmetic skin anti-ageing treatment, characterized in that it comprises the application, to the appropriate skin areas of a person in need thereof, of an effective amount of at least one substance promoting intercellular communication for obtaining an anti-ageing effect on said skin areas, especially for improving the firmness and elasticity of the skin, for delaying the appearance of wrinkles or for
- 10 reducing their depth, and particularly a substance promoting intercellular communication as defined above.

The Examples which follow are given purely to illustrate the invention with reference to the attached drawings, in which:

- Figure 1 shows the curve representing the modulation of intercellular
- 15 communication as a function of donor age. It is obtained from keratinocytes isolated from donors of different ages by the so-called scrape-loading method. The curve is expressed as the ratio of the fluorescent surface area to the total surface area on the ordinate as a function of the donor age in years on the abscissa. "r" is the correlation coefficient, which in this case is equal to 0.76. The measurements
- 20 obtained with young donors are represented by filled squares with the standard deviation and the measurements denoted by blank circles were obtained with old donors, again with the standard deviation.

This Figure should be read in conjunction with Table I (Example I).

- Likewise, Figure 2 again shows a curve representing the modulation of
- 25 intercellular communication as a function of donor age, obtained by a microinjection method, except that this time the curve is expressed as the number of labeled keratinocytes on the ordinate as a function of the age expressed in years on the abscissa. r again represents the correlation coefficient, which in this case is equal to 0.91. The measurements obtained with young donors are denoted by black squares
- 30 with their standard deviation and the measurements obtained with old donors are denoted by blank circles with their standard deviation.

This Figure should be read in conjunction with Table II (Example I).

- Figure 3 shows the change in the level of connexin 43 measured on
- 35 keratinocytes of donors of different ages by flux cytometry and expressed as the percentage labeling on the ordinate as a function of the donor age (in years) on the

abscissa, with a correlation coefficient r , which in this case is equal to 0.82. The measurements obtained with young donors are denoted by black squares with their standard deviation and the measurements obtained with old donors are denoted by blank circles with their standard deviation.

5 This Figure should be read in conjunction with Table III (Example I).

- Figure 4 shows the results of modulation of the intercellular communication of normal human keratinocytes, denoted by NHK, of different donors who may or may not have been treated with a lipid extract of the alga *Skeletonema costatum*, abbreviated to SKC, the results being represented in the form of histograms and expressed as relative diffusion units on the ordinate, the blank bar being obtained with control NHK and the shaded bar being obtained with NHK treated in vitro with a lipid extract of the alga *Skeletonema costatum* in a proportion of 2.5 $\mu\text{g/ml/24 h}$.

This Figure should be read in conjunction with Table VI (Example III).

- Figure 5 shows the measurement, by the microinjection technique, of the modulation of the intercellular communication of normal human keratinocytes (abbreviated to NHK) of different donors who may or may not have been treated with a lipid extract of the alga *Skeletonema costatum*, abbreviated to SKC, with the number of labeled cells on the ordinate, the results with the untreated, control NHK being shown by a blank bar marked "control", and the NHK treated with a lipid extract of the alga *Skeletonema costatum*, or SKC, at a dose of 2.5 $\mu\text{g/ml/24 h}$ being shown by a black bar marked "treated".

This Figure should be read in conjunction with Table V (Example III).

- Figure 6 shows another histogram, obtained from results measured by flux cytometry, of the modulation of the amount of connexin 43, relative to their respective controls, in donors of different ages treated with the lipid extract of the alga *Skeletonema costatum*, abbreviated to SKC, at a dose of 2.5 $\mu\text{g/ml/24 h}$, with the percentage increase relative to their respective controls on the ordinate and the donor age in years on the abscissa.

This Figure should be read in conjunction with Table VI (Example III).

30 Other advantages of the invention will become apparent from the description and Examples which follow.

Unless indicated otherwise, the proportions given in the Examples of compositions are expressed as percentages by weight.

35 **Example I - Demonstration of the decrease in functionality of the gap junctional intercellular communication (GJIC) with age and the decrease in**

the amount of connexin 43 with age

1.1- Materials and methods

5 1.1.1- Culture of normal human keratinocytes

The cultures of normal human keratinocytes (NHK) are prepared from skin samples.

10 The sample is first rinsed 4 times in PBS (Phosphate Buffered Saline - Sigma) (50 ml tubes). It is then decontaminated by successive immersion in two baths of 70% ethanol for 30 seconds. When the sample has been decontaminated, it is placed in a Petri dish containing PBS. 1 mm wide strips are then cut out, care being taken to remove as much fatty tissue and dermis as possible. The strips are placed immediately in a Petri dish containing PBS.

15 For recovery of the keratinocytes from the epidermis, the strips are placed for 4 h at 37°C in a 0.25% solution of trypsin in PBS.

The dermis is then separated from the epidermis by scraping the strips with a scalpel, the epidermal cells obtained being suspended in a tube containing DMEM (Dulbecco Modified Eagle's Medium - Gibco) + 10% FCS (Fetal Calf Serum - Eurobio). After homogenization of the suspension, the surface portion consisting of stratum corneum cells is removed and the remaining suspension is filtered on a sieve.

20 The filtered portion is centrifuged for 5 minutes at 176 g. The residue is taken up with NHK-D medium (DMEM + 10% FCS + 0.4 µg/ml hydrocortisone + 10 ng/ml EGF + 10^{-9} M cholera toxin). The cells are counted and then inoculated at a rate of 15×10^6 cells/flask.

After 24 h of culture, the medium is changed, the cells are rinsed with PBS and K-SFM proliferation medium (Gibco) is used for the remainder of the culture.

The keratinocytes are subcultured in totally conventional manner, but they have to be subcultured at 60-70% of confluence in order to retain their capacity to proliferate. Thus, when the cells are at 60-70% of confluence, the maintenance medium is removed and the cellular mat is rinsed with PBS. The cells are placed in 3 ml of trypsin/EDTA solution; then, when the cells detach, the trypsin is inhibited with a medium containing 10% of FCS (Eurobio). The cell suspension is homogenized, recovered and then centrifuged at 20 g for 5 minutes at room temperature. The resulting residue is taken up with medium only. For

maintenance, inoculation is carried out as before at a rate of 10^6 cells/75 cm² in ventilated flasks stored under the conditions mentioned above. Confluence is obtained after about ten days and the cells can be amplified over 6 to 7 passes.

- In the case where it is desired to test the activity of a substance on these
- 5 keratinocytes, the tests will be performed using the culture medium containing keratinocytes at the moment of confluence described above.

1.1.2- Scrape-loading

- This technique was developed in 1987 by Trosko's group (El-Fouly, M.H.,
- 10 Trosko, J.E. & Chang, C.-C. (1987). Scrape loading and dye transfer - a rapid and simple technique to study gap junctional intercellular communication. *Experimental Cell Research*, **168**, 422-430). It is based on the use of the dye Lucifer Yellow (Sigma) of molecular weight (MW) 457.2. This molecule (an aminophthalimide) is highly fluorescent and only diffuses through the gap junctions. It does not pass
 - 15 through the plasma membrane of intact cells. The cellular layer is injured with a scalpel, allowing the dye access to the gap junctions.

- The cells at confluence are rinsed with PBS (Sigma) before the dye is added. 2 ml of 0.05% Lucifer Yellow (Sigma) diluted in PBS are deposited on the cells and the scrape is carried out at room temperature. Six scrapes are carried out in 60 mm
- 20 dishes. The cells are left in contact with the dye for 5 minutes, the latter then being withdrawn and recovered. The cells are then rinsed with PBS to remove the excess fluorescence. They are fixed with 3% paraformaldehyde (PFA) for 10 minutes. The cells are rinsed again with PBS, 2 ml of PBS being left in the dishes after rinsing.
 - 25 The dishes are then observed with an inverted fluorescence microscope fitted with an image acquisition camera. Six or eight photographs are taken for each dish and then analyzed with an image analyzing software (Perfectimage, Iconix). The fluorescence corresponding to the surface area occupied by the Lucifer Yellow in the cellular layers is then quantified. This is done by placing a
 - 30 rectangle of predefined size in the zone to be studied. The computer calculates the surface area ratio: fluorescent surface area/total surface area. The higher the ratio, the greater is the capacity of the cells to communicate with one another.

I.1.3- Microinjection

The cells are prepared in a manner similar to that used by the scrape-loading technique.

The precision of this technique allows the Lucifer Yellow (10% in 0.33 M LiCl) to be injected directly into a cell solely by means of a capillary connected to a micromanipulator (Leitz). The injection is controlled by an automatic injector (Tranjector 5246, Eppendorf) and lasts 1 second with an intensity of 10,335 HPa. The injections are performed under an inverted microscope (20X) in the phase contrast mode (Leica microscope, Fluovert FU). About twenty injections are performed in a 60 mm Petri dish.

After the cells have been fixed with 3% paraformaldehyde, they are rinsed with PBS. This is followed by observation and counting of the number of labeled cells.

I.1.4- Flux cytometry

When confluence is reached, the culture medium is removed and the cells are rinsed twice with PBS. They are then trypsinized with a mixture of 0.1% trypsin and 0.02% EDTA. Inhibition of the trypsin is effected with culture medium supplemented with 10% of FCS. The number of cells is counted so that it can be adjusted to 10^6 cells per tube. The cell suspension is centrifuged at 176 g for 5 minutes and the residue is taken up with 100 ml of PBS. The cells are fixed with 3% PFA for 25 minutes at 4°C. To remove the PFA, the cells are centrifuged again at 176 g for 5 minutes and rinsed twice in PBS.

The buffer in which the antibodies are prepared is composed of PBS, 1% of FCS and 0.2% saponin (Sigma), a detergent which induces microporation of the membrane but does not damage the cell. This detergent is used to keep both the intracytoplasmic and the transmembrane connexin 43 intact and quantify it (personal communication, R. Mouawad, Hôpital Salpêtrière - Paris).

The primary antibody used is anti-connexin 43 (Zymed, monoclonal antibody created in the mouse). The concentration of the primary antibody must be 1.3 µg/ml (1/750). Incubation is carried out for 45 minutes at 4°C. To remove the excess antibody, the cells are centrifuged and rinsed with the buffer used to prepare the antibodies (PBS, saponin and FCS). They are then brought into contact with the secondary antibody, a fluorescein-coupled anti-mouse antibody, at a dilution of 1/50 (Jackson Immunotech). After incubation with the second antibody, the cells

are centrifuged and rinsed and then taken up with 1 ml of buffer (PBS, saponin, FCS).

The fluorescence intensity in the NHK treated as described above and in NHK which have not been brought into contact with the fluorescein-coupled anti-mouse secondary antibody is measured with a flux cytometer (Epics profile II from COULTRONIC) and the measurements are processed and compared by a software (Phoenix flow system soft from COULTRONIC), which provides a value measured in an arbitrary unit given by the machine. This is called the level of labeling of the connexin 43 in the NHK, which reflects the amount of connexin 43 per NHK (averaged).

1.2- Results

1.2.1- Evaluation of the functionality of gap junctional intercellular communication (GJIC) as a function of donor age

1.2.1.1- Scrape-loading (Figure 1)

The study was conducted on 9 donors aged between 40 and 75 years.

Table I

Modulation of intercellular communication as a function of donor age

Donor age (years)	Fluorescent surface area/total surface area (mean)	Standard deviation
40	0.41	0.06
41	0.40	0.05
43	0.60	0.07
47	0.33	0.14
48	0.10	0.08
67	0.31	0.06
68	0.33	0.08
70	0.25	0.05
75	0.21	0.04

From the results shown in Table I and Figure 1 (attached), it can be seen that intercellular communication decreases with donor age, the capacity of the keratinocytes to communicate apparently being inversely proportional to donor age.

The correlation coefficient is 0.76.

I.2.1.2- Microinjections (Figure 2)

The study was conducted on the same donors as for the scrape-loading, with the exception of the 43-year-old donor.

Table II

Modulation of intercellular communication as a function of donor age

Donor age (years)	Number of labeled cells (mean)	Standard deviation
40	15.1	3.6
41	16.9	5.4
47	11.8	3.2
48	10.1	2.2
67	8.6	2.7
68	8.7	2.6
70	5.9	2.4
75	6.8	1.7

From the results shown in Table II and Figure 2 (attached), we see that GJIC is inversely proportional to age; however, with this technique, which is more precise than scrape-loading, the correlation coefficient is 0.91.

The two techniques, scrape-loading and microinjections, give results which show the same trend: the functionality of GJIC decreases very significantly with age.

I.2.2- Evaluation of the effect of age on the amount of connexin 43 (Figure 3)

Flux cytometry

The study was conducted on the same donors as for the scrape-loading, with the exception of the 43-year-old donor and the 75-year-old donor.

Table III

Level of labeling of connexin 43 in NHK, measured on keratinocytes of donors of different ages

Donor age (years)	Level of labeling of connexin 43 in NHK (mean)	Standard deviation
40	55.1	4.7
41	54.5	7.5
47	42.1	2.5
48	28.9	1.8
67	19.1	0.8
68	27.1	7.1
70	25.6	2.0

It is seen from Table III and Figure 3 (attached) that the level of connexin 43 decreases with age, the amount of connexin 43 apparently being inversely proportional to donor age.

The correlation coefficient is 0.82.

These results show that there is an ageing effect on the functional capacity of cells to communicate with one another, especially via the gap junctions (GJ), and an ageing effect on the amount of connexin 43 present in the cells. In fact, they show that the functional capacity of cells to communicate with one another, especially via the gap junctions (GJ), decreases with age. They also show that the amount of connexin 43 decreases with age.

The inventors have therefore shown the value of using substances which promote intercellular communication, especially via the GJ, for combating the signs of skin ageing and particularly for slowing down their appearance. They have therefore developed a novel means of combating skin ageing, particularly wrinkles and the loss of elasticity of the skin.

The inventors have therefore also shown the value of using substances which promote intercellular communication, especially via the GJ, for promoting and/or increasing the activity of a cosmetic agent. They have therefore developed a novel means of promoting and/or increasing the activity of a cosmetic agent.

Example II - Preparation of lipid extracts of the alga *Skeletonema costatum*

II.1- Extraction with isopropanol by a first method

Preferably, the whole extraction will be performed under an inert atmosphere (nitrogen saturation) in order to avoid pronounced degradation of the active molecules.

250 kg of biomass (*Skeletonema costatum*) are used in this preparation.

The algae, which have been frozen to -20°C, are immersed in isopropanol (IPA) refluxing at 80-83°C, with agitation. The thermal shock makes it possible to facilitate the decantation of the silica (originating from the skeleton of the algal cells).

The amount of solvent used is 10 liters of IPA per liter of water present in the biomass. In this preparation, for a proportion of dry matter of 30%, the 250 kg of biomass represent 75 kg of dry matter and 175 kg of water. The amount of IPA used here is 1925 kg.

The whole (biomass + IPA) is refluxed for half an hour at about 80°C, with agitation, before being cooled to about 50°C.

After the reaction mixture has been cooled to about 50°C, the extract is transferred to a GUEDU filter in order to separate the extracted biomass from the IPA lipid extract.

The lipid extract is concentrated in a batch reactor (concentration factor = 71.5).

The yield of crude oil in this first step is 28%, based on dry weight.

To start the second step, the lipid extract is taken up with cold IPA at a rate of 10 kg of solvent per kg of oil. Agitation is continued for 20 minutes. The liquor is then filtered (enabling the residual sticky sludge to be removed).

The decolorization and deodorization treatment is carried out in two batches in an 80-liter Schott reactor and takes 30 minutes at room temperature. 0.94 kg of zeolite (ABSENT 2000 supplied by UOP) and 1.6 kg of active charcoal (CXV supplied by CECA) are added, the charcoal-to-zeolite ratio being 1.7.

The zeolite and charcoal are then filtered off on paper.

The yield of this second step is 37%, based on dry weight.

Thus the overall yield of oil for the process as a whole is 10%, based on dry weight of biomass.

Antioxidants (DL- α -tocopherol at a final concentration of 0.05% by weight

and ascorbyl palmitate at a final concentration of 0.05% by weight) are incorporated by way of a stock solution in IPA.

The filtrate and antioxidants are then concentrated batchwise to give a brown-colored oil.

- 5 This lipid extract (in the form of an oil) is packaged under an inert gas such as nitrogen.

This oil will be referred to below as SKC lipid extract E1.

II 2- Extraction with ethanol by a second method

- 10 The extraction begins with the dispersion of 49.8 kg of frozen vegetable biomass (29% of dry matter) in 539 kg of 96% ethanol alkalized with 9 kg of 30.5% aqueous sodium hydroxide solution. After maceration for 30 minutes at 40°C under reflux and under a nitrogen atmosphere, the whole is cooled to 18°C.

The insoluble materials are then filtered off under nitrogen and removed.

- 15 151 kg of distilled water are added to the 573.9 kg of filtrate. The whole is agitated slowly for 10 minutes before being added to 162 kg of heptane. The heptane epiphase of the liquid-liquid partition is removed. It is pointed out that the hypophase contains the fatty acids in the form of salts. The operation is repeated two more times.

- 20 The 756 kg of solution constituting the above-mentioned hypophase are acidified by the addition of 2.8 kg of sulfuric acid to give a pH of 2.2. The whole is agitated for 10 minutes under nitrogen before being added to 158 kg of heptane. The free fatty acids are extracted from the 147 kg constituting the first heptane epiphase of this new liquid-liquid partition. The operation is repeated five more
25 times to recover a total of 697 kg of heptane phase. This phase is evaporated to dryness on a rotary evaporator and then by molecular distillation to give the active extract in an amount representing 1.1 kg of oil.

The oil produced is a dark yellow-colored homogeneous liquid.

This oil will be referred to below as SKC lipid extract E2.

30

Example III - Evaluation of the effect of a lipid extract of the alga SKC on gap junctional intercellular communication (GJIC) and the level of connexin 43 in NHK of old donors

- 35 The lipid extract of the alga SKC used in this Example is SKC lipid extract E2 obtained by the ethanol extraction method described above.

The studies described below were also conducted with SKC lipid extract E1 (results not shown), the results being comparable to those described below and obtained with SKC lipid extract E2.

The studies are conducted on normal human keratinocytes (NHK) obtained according to the protocol described in Example I (I.1.1 - Culture of normal human keratinocytes). In contrast to the controls, the NHK treated with SKC lipid extract E1 or E2 (at the moment of confluence of the culture) are brought into contact with an amount "x" of said extract, expressed in μg per milliliter of culture medium per 24 hours ($x \mu\text{g/ml/24 h}$), before being evaluated by the techniques of scrape-loading, microinjections or flux cytometry according to the protocols described in Example I (I.1.2, I.1.3 and I.1.4 respectively).

• Scrape-loading (Figure 4)

The study was conducted on normal human keratinocytes (NHK) of 4 donors aged between 60 and 79 years.

Table IV

Modulation of the intercellular communication of NHK of different donors treated with SKC lipid extracts (E2), measured by the scrape-loading method

Donor		Treatment and dose			
		SKC lipid extracts (E2)			Control
Age	Data	1.25 $\mu\text{g/ml}$	2.5 $\mu\text{g/ml}$	5 $\mu\text{g/ml}$	0
63 years	R (mean)	0.55	0.53	0.50	0.38
	Stand. dev.	0.05	0.07	0.04	0.07
79 years	R (mean)	0.53	0.54	0.59	0.42
	Stand. dev.	0.06	0.06	0.11	0.07
60 years	R (mean)	0.51	0.54	0.57	0.38
	Stand. dev.	0.07	0.06	0.05	0.06
73 years	R (mean)	0.61	0.56	0.65	0.49
	Stand. dev.	0.05	0.07	0.07	0.04

"R" is the ratio of the fluorescent surface area to the total surface area.

The results shown in Table IV and Figure 4 (attached) demonstrate that the SKC lipid extracts induce an increase in GJIC in old donors ($p < 0.0001$ - Student

test).

The SKC lipid extracts therefore restore cellular communication to approximately the same level as in NHK of young donors.

5 • Microinjections (Figure 5)

The study was conducted on 12 donors aged between 49 and 79 years.

Table V

Modulation of the intercellular communication of NHK of different donors treated with SKC lipid extracts (E2)

5

Donor		Treatment and dose	
		SKC lipid extract (E2)	Control
Age	Data	2.5 µg/ml	0
49 years	Number of labeled cells (mean)	15.9	7.7
	Standard deviation	1.5	2.0
52 years	Number of labeled cells (mean)	21.4	10.9
	Standard deviation	2.6	2.1
55 years	Number of labeled cells (mean)	19.0	8.7
	Standard deviation	2.2	1.2
55 years	Number of labeled cells (mean)	13.9	8.6
	Standard deviation	2.8	2.1
59 years	Number of labeled cells (mean)	13.1	5.3
	Standard deviation	1.7	0.9
59 years	Number of labeled cells (mean)	23.4	11.0
	Standard deviation	4.3	1.9
60 years	Number of labeled cells (mean)	12.4	8.4
	Standard deviation	1.7	1.9
61 years	Number of labeled cells (mean)	13.8	8.1
	Standard deviation	1.4	1.8
62 years	Number of labeled cells (mean)	19.6	10.0
	Standard deviation	3.9	2.9
65 years	Number of labeled cells (mean)	23.9	10.9
	Standard deviation	3.5	2.7
71 years	Number of labeled cells (mean)	9.5	4.1
	Standard deviation	1.3	1.2
79 years	Number of labeled cells (mean)	13.9	8.3
	Standard deviation	1.9	1.8

The number of labeled cells is seen to increase by a factor of 2 ($p < 0.0001$ - Student test). The SKC lipid extracts therefore induce a restoration of the GJIC of cells of old donors to approximately the level of the GJIC of cells of young donors.

• Flux cytometry (Figure 6)

The study was conducted on 6 donors aged between 49 and 60 years.

Table VI

5

Modulation of the amount of connexin 43 after treatment with SKC lipid extract (E2) at 2.5 µg/ml/24 h, measured by flux cytometry

Donor age (years)	Percentage increase in the amount of connexin 43 relative to the respective controls (%)
49	31.5
52	25.5
55	30.8
59	11.5
59	19.0
60	35.2

10

A mean increase of 25.6% in the labeling of the treated cells relative to the control cells is observed.

The SKC lipid extracts induce an increase in the amount of connexin 43.

15

The results obtained with the different means of evaluating the effect of lipid extracts of the alga *Skeletonema costatum* on intercellular communication, especially via the GJ, and on the level of connexin 43, carried out on NHK of old donors, show that the use of lipid extracts of the alga *Skeletonema costatum* makes it possible to restore intercellular communication, especially via the GJ, and also to increase the level of connexin 43 present in the NHK.

20

These results show the value of using lipid extracts of the alga *Skeletonema costatum* in cosmetic compositions for combating the signs of skin ageing and particularly for slowing down their appearance. The inventors have therefore developed a novel means of combating skin ageing, particularly wrinkles and the loss of elasticity of the skin.

25

Furthermore, the results obtained above show the value of using lipid extracts of the alga *Skeletonema costatum* in cosmetic compositions for promoting and/or increasing the activity of other cosmetic agents which act directly in the cell or via intracellular second messengers, and which may or may not be present in the same cosmetic composition as the SKC lipid extracts.

Example IV - Evaluation of the effect of boldine by the scrape-loading test

The study was conducted on normal human keratinocytes (NHK), the concentrations used being 12.5, 25, 50, 100 and 200 mM. The incubations take 24 h in the culture medium. The Table below summarizes the measured values of the surface area of diffusion.

Table VII

Concentration of boldine (mM)	control	15.5	25	50	100	200
Mean surface area of diffusion	0.46	0.47	0.51	0.574	0.512	0.39
Standard deviation	0.07	0.06	0.04	0.03	0.66	0.08

As can be seen in Table VII, the dose which seems to be the most effective for increasing the functionality of GJIC is 50 mM/24 h. Statistical analysis of the data shows that only this concentration affords a really significant difference in the increase in GJIC relative to the untreated NHK. The results in Table VII suggest that boldine has a dose-related effect on GJIC.

Example V - Anti-ageing day cream for the face

Glyceryl stearate + PEG-100 stearate	6.00 %
Squalane	3.00 %
Hydrogenated polyisobutene	3.00 %
Glycerol tricaprylate/caprate	3.00 %
Glycerol	2.00 %
Octyl methoxycinnamate	2.00 %
Beeswax	1.50 %
Cetostearyl octanoate	1.50 %
Cetyl alcohol	1.00 %
Stearyl alcohol	1.00 %
Dimethicone	1.00 %
SKC lipid extract E2	1.00 %
Xanthan gum	0.20 %
Carbomer	0.15 %
Neutralizing agent	qs
Preservatives	qs
Perfume, colors	qs
Water	qsp 100.00 %

Example VI - Anti-wrinkle emulsion-gel for the face

Glycerol	5.00 %
Caprylic/capric/succinic triglycerides	3.00 %
SKC lipid extract E2	2.00 %
Octyl methoxycinnamate	1.00 %
Acrylates/C10-30 alkyl acrylate crosspolymer	0.50 %
Wheat protein hydrolyzate	0.50 %
Dimethicone copolyol	0.50 %
Neutralizing agent	qs
Preservatives	qs
Perfume, colors	qs
Water	qsp 100.00 %

Example VII - Firming emulsion for the body

5

Octyl palmitate	7.00 %
Glycerol tricaprylate/caprate	3.00 %
Octyl octanoate	2.00 %
Phenyltrimethicone	2.00 %
Glycerol	2.00 %
Stearic acid	1.00 %
Sorbitan stearate, 20 EO	0.90 %
Cetyl alcohol	0.50 %
Stearyl alcohol	0.50 %
SKC lipid extract E2	0.50 %
Carbomer	0.40 %
Xanthan gum	0.20 %
Sorbitan stearate	0.10 %
Neutralizing agent	qs
Preservatives	qs
Perfume, colors	qs
Water	qsp 100.00 %

Example VIII - Anti-wrinkle emulsion-gel for the face

Glycerol	5.00%
Caprylic/capric/succinic triglycerides	3.00%
Octyl methoxycinnamate	1.00%
Acrylates/C10-30 alkyl acrylate crosspolymer	0.50%
Wheat protein hydrolyzate	0.50%
Dimethicone copolyol	0.50%
Boldine	0.01%
Neutralizing agent	qs
Preservatives	qs
Perfume, colors	qs
Water	qsp 100.00%

CLAIMS

1. Cosmetic composition, characterized in that it comprises, as an active ingredient, at least one substance which promotes the intercellular communication of skin cells, particularly keratinocytes, fibroblasts and skin preadipocytes.
2. Composition according to claim 1, characterized in that said substance promotes intercellular communication via the gap junctions of skin cells, particularly keratinocytes, fibroblasts and skin preadipocytes.
3. Composition according to claim 1 or 2, characterized in that said substance which promotes intercellular communication promotes the formation of connexin, particularly connexin 43.
4. Composition according to one of claims 1 to 3, characterized in that said substance which promotes intercellular communication comprises at least one lipid extract of the alga *Skeletonema*, especially the alga *Skeletonema costatum*, and particularly a total lipid extract of said alga.
5. Composition according to claim 4, characterized in that said extract is obtained by extracting the alga *Skeletonema* with an alcoholic solvent selected from the group consisting of isopropanol, ethanol and methanol.
6. Composition according to claim 5, characterized in that the above-mentioned extract is obtained by extracting the alga with isopropanol.
7. Composition according to claim 5, characterized in that the above-mentioned extract is obtained by extracting the alga with ethanol.
8. Composition according to one of claims 4 to 7, characterized in that the extraction is performed under reflux.
9. Composition according to one of claims 4 to 8, characterized in that the alga is frozen before being extracted with the alcoholic solvent, the freezing preferably being effected at a temperature of between about -40°C and -20°C and for a period preferably of between about 1 and 7 days.
10. Composition according to one of claims 4 to 9, characterized in that the frozen alga is immersed directly in the heated alcoholic solvent.
11. Composition according to one of claims 5 to 10, characterized in that the above-mentioned algal extract is obtained after the following series of steps:
 - a) the alcoholic solvent is alkalinized to a pH of between 10 and 14, preferably to a pH of 13, for example with aqueous sodium hydroxide solution or aqueous potassium hydroxide solution,

- b) the insoluble materials are removed from the aqueous-alcoholic phase,
 - c) distilled water is added to the aqueous-alcoholic phase,
 - d) the solution obtained is subjected to liquid-liquid extraction with an apolar solvent immiscible with the aqueous-alcoholic phase, for example heptane,
5 hexane or cyclohexane,
 - e) the phase containing the apolar solvent is removed,
 - f) the aqueous-alcoholic phase recovered after removal of the phase containing the apolar solvent is acidified to a pH of between 1 and 3, preferably to a pH of 2, for example with aqueous sulfuric acid solution or aqueous hydrochloric acid
10 solution,
 - g) the solution obtained after acidification is subjected to liquid-liquid extraction with an apolar solvent immiscible with the aqueous-alcoholic phase, for example heptane, hexane or cyclohexane,
 - h) the aqueous-alcoholic phase is removed, and
 - 15 i) the phase containing the apolar solvent recovered after removal of the aqueous-alcoholic phase is evaporated to give an oil free of apolar solvent, this oil being the target extract.
12. Composition according to claim 4, characterized in that the above-mentioned extract is obtained by extraction with supercritical CO₂.
- 20 13. Composition according to one of claims 5 to 11, characterized in that, before any extraction operation, the alga is macerated in the alcoholic solvent at room temperature, preferably for a period of between about 5 minutes and 80 minutes and particularly preferably for a period of between about 20 minutes and 40 minutes.
- 25 14. Composition according to one of claims 5 to 10 or 13, characterized in that the amount of alcoholic solvent used is between about 0.1 liter and 20 liters of solvent, preferably between about 2 liters and 10 liters of solvent, per 100 g of alga, expressed by dry weight of alga.
- 30 15. Composition according to one of claims 4 to 14, characterized in that the extraction is performed under an inert atmosphere, preferably a nitrogen-saturated atmosphere.
16. Composition according to one of claims 4 to 15, characterized in that it comprises from about 0.01% to 10% and particularly from about 0.1% to 3% by weight of said lipid extract of the alga *Skeletonema*, especially the alga *Skeletonema* costatum, based on the total weight of the final composition.
- 35

17. Composition according to one of claims 1 to 3, characterized in that said substance which promotes the intercellular communication of skin cells is boldine.
18. Composition according to claim 17, characterized in that it comprises from about 0.001% to 10% and particularly from about 0.01% to 1% by weight of boldine, based on the total weight of the final composition.
19. Use of at least one substance which promotes the intercellular communication of keratinocytes, fibroblasts and skin preadipocytes as a cosmetic agent, optionally in the presence of a cosmetically acceptable vehicle.
20. Use according to claim 19, characterized in that said substance promotes intercellular communication via the gap junctions of keratinocytes, fibroblasts and skin preadipocytes.
21. Use according to claim 19 or 20, characterized in that the substance which promotes intercellular communication promotes the formation of connexin, particularly connexin 43.
22. Use according to one of claims 19 to 21, characterized in that said substance comprises at least one lipid extract of the alga *Skeletonema*, especially the alga *Skeletonema costatum*, and particularly a total lipid extract of said alga, especially as defined in any one of claims 5 to 16, said extract advantageously being obtained by liquid-liquid extraction between an alkalinized and then acidified alcohol and an apolar solvent immiscible with the aqueous-alcoholic phase, for example heptane, hexane or cyclohexane.
23. Use according to one of claims 19 to 21, characterized in that said substance is boldine.
24. Method of promoting and/or increasing the activity of a cosmetic agent acting directly in the cell or via intracellular second messengers, characterized in that it comprises the application, simultaneously with or prior to that of said cosmetic agent, to the appropriate skin areas of a person in need thereof, of an effective amount of at least one substance promoting intercellular communication, particularly a substance promoting intercellular communication as defined in any one of claims 2 to 18.
25. Method of cosmetic skin anti-ageing treatment, characterized in that it comprises the application, to the appropriate skin areas of a person in need thereof, of an effective amount of at least one substance promoting intercellular communication for obtaining an anti-ageing effect on said skin areas, especially for improving the firmness and elasticity of the skin, for delaying the appearance of

FIG.1

MODULATION OF INTERCELLULAR COMMUNICATION AS A
FUNCTION OF DONOR AGE , MEASURED BY SCRAPE-LOADING

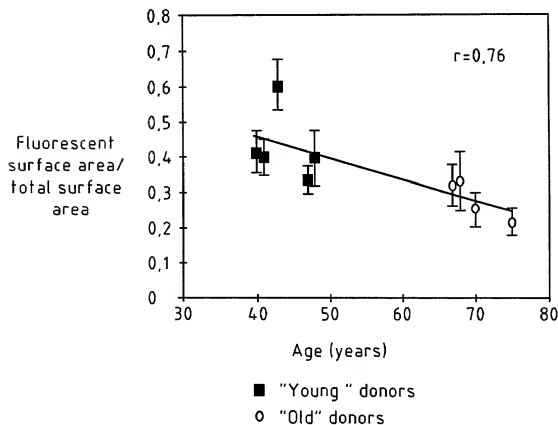


FIG.2

MODULATION OF INTERCELLULAR COMMUNICATION AS A
FUNCTION OF DONOR AGE , MEASURED BY MICROINJECTION

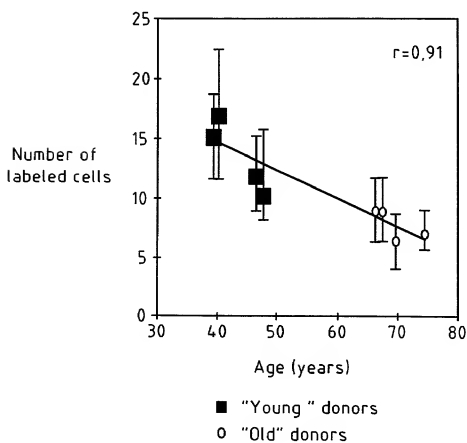


FIG.3

CHANGE IN THE LEVEL OF CONNEXIN 43 MEASURED ON
KERATINOCYTES OF DONORS OF DIFFERENT AGES
BY FLUX CYTOMETRY

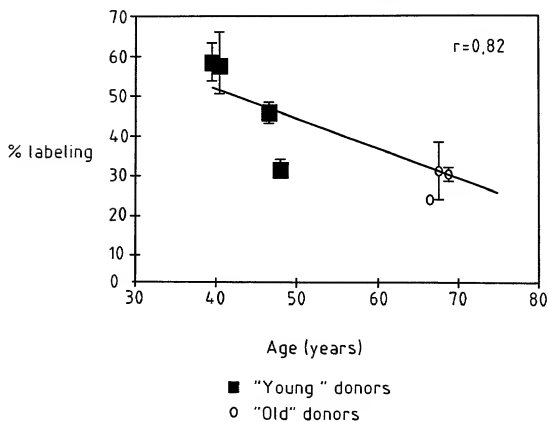


FIG.4

MODULATION OF THE INTERCELLULAR COMMUNICATION OF
NHK OF DIFFERENT DONORS TREATED WITH A LIPID EXTRACT OF THE
ALGA SKC, MEASURED BY SCRAPE-LOADING

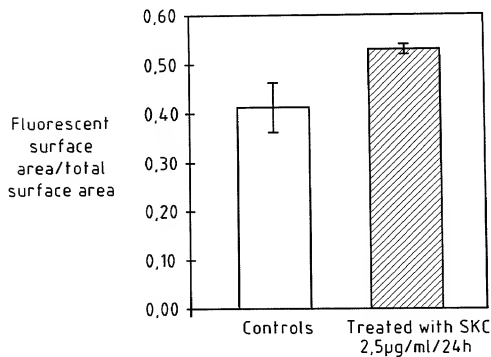


FIG.5

MODULATION OF THE INTERCELLULAR COMMUNICATION OF
NHK OF DIFFERENT DONORS TREATED WITH AN SKC LIPID EXTRACT ,
MEASURED BY MICROINJECTION

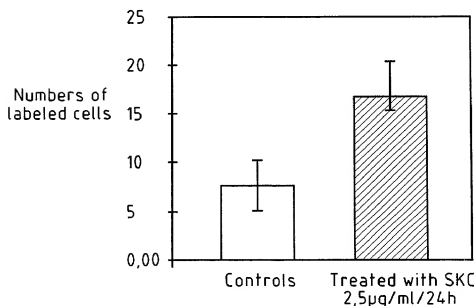
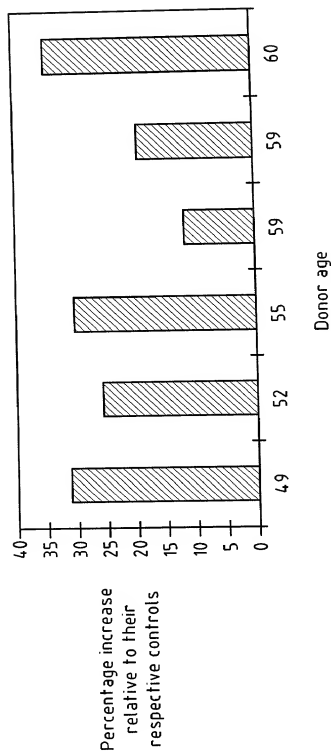


FIG.6

MODULATION OF THE AMOUNT OF CONNEXIN 43 AFTER
TREATMENT WITH THE SKC LIPID EXTRACT AT
2.5 $\mu\text{g}/\text{ml}$ 24h, MEASURED BY FLUX CYTOMETRY



COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
(Includes Reference to PCT International Applications)ATTORNEY'S DOCKET NUMBER
24795

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Cosmetic composition comprising at least one substance promoting the formation of
connexin, use and method of cosmetic treatment

the specification of which (check only one item below):

☐ is attached hereto.☒ was filed as United States applicationSerial No. 09/937848on September 28, 2001,

and was amended

on _____ (if applicable).

☒ was filed as PCT international applicationNumber PCT/FR00/00818on March 31, 2000,

and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (If PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
France	99 04165	02/04/1999	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

Combined Declaration For: Patent Application and Power of Attorney (Continued)

(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER
24795

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS

STATUS (Check one)

U.S. APPLICATION NUMBER

U.S. FILING DATE

PATENTED

PENDING

ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.

PCT APPLICATION NO.

PCT FILING DATE

U.S. SERIAL NUMBERS
ASSIGNED (if any)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (List name and registration number)
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	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	SECOND GIVEN NAME
202	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	SECOND GIVEN NAME
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	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	SECOND GIVEN NAME

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
DATE	DATE	DATE

204	FULL NAME OF INVENTOR	FAMILY NAME	KRZYCH	FIRST GIVEN NAME	Valérie	SECOND GIVEN NAME
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	POST OFFICE ADDRESS	POST OFFICE ADDRESS	210 Chemin de la Pucelle	CITY	MENESTREAU en VILLETTE	STATE & ZIP CODE/COUNTRY
206	FULL NAME OF INVENTOR	FAMILY NAME		FIRST GIVEN NAME		SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY		STATE OR FOREIGN COUNTRY		COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS		CITY		STATE & ZIP CODE/COUNTRY
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SIGNATURE OF INVENTOR 204			SIGNATURE OF INVENTOR 205		SIGNATURE OF INVENTOR 206	
DATE December 21, 2001			DATE December 21, 2001		DATE	